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N-2-(2,4-Dinitrophenyl)ethyloxycarbonyl-Amino Acids, New Base Labile Protected Derivatives Suitable for Solid-Phase Peptide Synthesis.[1]

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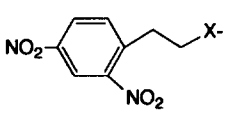
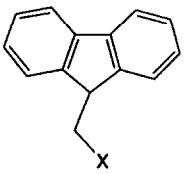
Abstract : The base labile N-2-(2,4-dinitrophenyl)ethyloxycarbonyl (Dnpeoc) group has been developed for the protection of the α-amino group of amino acids. Preparation of Dnpeoc-amino acids and their application to solid-phase peptide synthesis are described.

Solid-phase peptide synthesis has become the most used technique for the preparation of biologically active peptides and related compounds. Two strategies are commonly being used: the Boc/Bzl strategy that uses the acid-labile *t*-butyloxycarbonyl (Boc) group for the protection of the amino group and the Fmoc/tBu strategy that utilizes the base-labile 9-fluorenylmethyloxycarbonyl (Fmoc) as amino protecting group[3]. The base labile protecting group (Fmoc) allows the preparation of peptides in milder conditions because side chain protecting groups and the peptide-solid support bond are more labile to acids than the side chain protecting groups used in the Boc/Bzl strategy. Furthermore, the Fmoc-protecting group makes possible to monitor the synthesis by measuring the absorbance of the *N*-fluorenylmethyl piperidine released during the deprotection of the Fmoc group[4]. On the other hand, certain problems have been assigned to Fmoc-amino acids like the low solubility of the derivatives that impedes the automation of the synthetic procedures, their higher cost and some side chain protection still not solved[5].

Figure 1 : Dnpe and Fm related protecting groups.



X= O,S Dnpe
X= OCO-NH Dnpeoc



X= O,S Fm
X= OCO-NH Fmoc

We have recently described the use of 2-(2,4-dinitrophenyl)ethyl (Dnpe)[6] for the protection of the thiol group of cysteine and 3-mercaptopropionic acid[7], we found that this group has similar chemical

characteristics to the Fm group and, in addition, Dnpe-derivatives have higher solubility as well as can be prepared from less costly starting materials. Based in this experience we have developed the *N*-2-(2,4-dinitrophenyl)ethoxycarbonyl (Dnpeoc) as protecting group for the α-amino function of amino acids[8].

The preparation of the Dnpeoc chloroformate and mixed carbonates is shown in figure 1. Starting from 2-phenylethyl acetate (1), 2-(2,4-dinitrophenyl)ethanol (2) was prepared essentially as described in ref. 6a. Reaction of this compound with phosgene in dichloromethane/toluene gave the chloroformate (3) in a 80% yield[9] that was reacted with *N*-hydroxysuccinimide and triethylamine in dioxane[10] yielding compound 4 (DnpeocOSu) in 50% yield[11]. Reaction of compound 2 with *p*-nitrophenyl chloroformate and pyridine in dichloromethane gave the mixed carbonate 5 (DnpeocONp) in 73% yield[12].

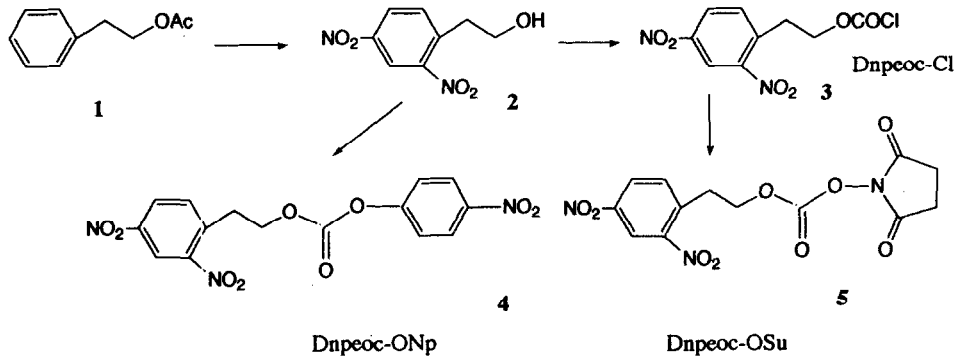


Figure 2 : Preparation of Dnpeoc acylating reagents.

Fluorenylmethyl succinimidyl carbonate (FmocOSu) has been shown to be the most suitable reagent for the preparation of Fmoc-amino acids, because reacts efficiently with free amino acids in dioxane-water mixtures in the presence of sodium carbonate without side products[13]. Using similar conditions low yields (20-30%) of Dnpeoc-amino acids were obtained using the corresponding succinimidyl carbonate (Dnpeoc-OSu) (see table 1). The low yields were assigned to a much lower reactivity of Dnpeoc-OSu compared with Fmoc-OSu. In the same conditions[13] but using the *p*-nitrophenyl carbonate (Dnpeoc-ONp), yields were higher (50-80%, see table 1) but still lower compared with those obtained with Fmoc-OSu. Besides, except for Dnpeoc-Asn and Dnpeoc-Gln, Dnpeoc-amino acids were difficult to separate from the *p*-nitrophenol generated in the reaction.

Table 1 : Preparation of Dnpeoc-amino acids. Yields obtained using compounds 3-5

	Dnpeoc-ONp 5	Dnpeoc-OSu 4	Dnpeoc-Cl 3
Dnpeoc-Gly	80%	26%	95%
Dnpeoc-Phe	62%	34%	88%
Dnpeoc-Leu	54%	20%	87%
Dnpeoc-Tyr(tBu)	46%	--	--
Dnpeoc-Asn	89%	--	--
Dnpeoc-Gln	48%	--	--
Dnpeoc-Ala	--	--	84%
Dnpeoc-Val	--	--	96%

Finally, when amino acids were treated with trimethylsilyl chloride followed by Dnpeoc-Cl[14] under the conditions described in ref. 14, the desired Dnpeoc-amino acids were obtained with excelent yields (84-95%, see table 1) and purity[15]. As described in ref. 14, the formation of the *O,N-bis*-trimethylsilyl-amino acids protects carboxyl function avoiding oligomerization and enhances the efficiency of acylation reactions. So, this method is recommended for the preparation of Dnpeoc-derivatives. The solubility of the Dnpeoc-derivatives in organic solvents is enormously increased compared with Fmoc-amino acids. For example, while Fmoc derivatives of Asn and Gln were only soluble in large amounts of DMF, the Dnpeoc derivatives of Asn and Gln were enough soluble in deuterochloroform to perform NMR spectra.

Deprotection of the Dnpeoc-derivatives has been studied both in solid-phase and in solution. Total elimination of the Dnpeoc group was found in less than 1 minute using the following conditions : a) 20% piperidine/ DMF; b) 0.5 M DBU in aprotic solvents (CH_2Cl_2, DMF, CH_3CN). The corresponding unprotected aminoacid together with 2,4-dinitrostyrene were the only products obtained when DBU was used for the deprotection. The solutions have a strong permanent blue color, assigned to the 2,4-dinitrostyrene anion. Using 20% piperidine, an extra 2,4-dinitrophenyl derivative was observed, that was assigned to the piperidine adduct of 2,4-dinitrostyrene by analogy to the Fmoc group. The conversion of the 2,4-dinitrostyrene to the piperidine adduct was slow (more than 2 hours) and it can be monitored as the strong blue color fades to the yellow-brown color of the piperidine adduct. On the other hand, Dnpeoc-derivatives were found to be stable in the solvent used in solid-phase peptide synthesis (DMF, CH_2Cl_2).

In order to test the use of the new derivatives for solid-phase peptide synthesis, the pentapeptide Leucine-enkephalinamide (H-Tyr-Gly-Gly-Phe-Leu-NH_2) was assembled manually on a Fmoc-PAL-polystyrene-resin[16]. DCC-mediated coupling reactions and standard Fmoc protocols were used, with the exception that three different syntheses were done using in each one a different deprotection solution: a) 20% piperidine/ DMF; b) 0.1M DBU in DMF; c) 0.1M DBU in CH_2Cl_2. In all three cases the deprotection of the Dnpeoc was easily monitored by the appearance of the blue color due to the anion of the 2,4-dinitrostyrene. Peptides were cleaved from resins with a TFA treatment giving, in all three cases, a product that coeluted with a known sample of Leu-enkephaline (purity >95% by analytical HPLC).

In conclusion, the preparation of new base labile protected derivatives (Dnpeoc-amino acids) suitable for solid-phase peptide synthesis is described. Our results indicates that these derivatives are a useful alternative to Fmoc-derivatives since i) they have better solubility properties, ii) their deprotection is faster and can be monitored and, iii) they are prepared from less costly starting materials.

REFERENCES AND NOTES

1. Abbreviations used are as follows: Boc: *t*-butyloxycarbonyl; Bzl: benzyl; DBU: 1,8-diazabicyclo [5.4.0]undec-7-ene; DCC: *N,N'*-dicyclohexylcarbodiimide; DIEA: *N,N*-diisopropylethylamine; DMAP: *N,N*-dimethylaminopyridine; DMF: N,N-dimethylformamide; Dnpe: 2-(2,4-dinitrophenyl)ethyl; Dnpeoc: 2-(2,4-dinitrophenyl)ethyloxycarbonyl; EtOH: ethanol; Fm: 9-fluorenylmethyl; Fmoc: 9- fluorenylmethyloxycarbonyl; Fmoc-PAL: [5-(4-((9-

fluorenylmethyloxycarbonyl)aminomethyl)-3,5-dimethoxy) valeric acid]; HOBt: 1-hydroxybenzotriazole; MeOH: methanol; tBu: *t*-butyl; TFA: trifluoroacetic acid.

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12. ^{1}H-NMR of compound **5** ($CDCl_3$, 90 MHz) : 8.80 (d, 1H); 8.45 (dd, 1H); 8.25 (d, 2H); 7.70 (d, 1H); 7.35 (d, 2H); 4.62 (t, 2H); 3.50 (t, 2H).
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